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EFFECT OF SMALL-PARTICLE AEROSOLS OF RIMANTADINE AND RIBAVIRIN --ETC(U)
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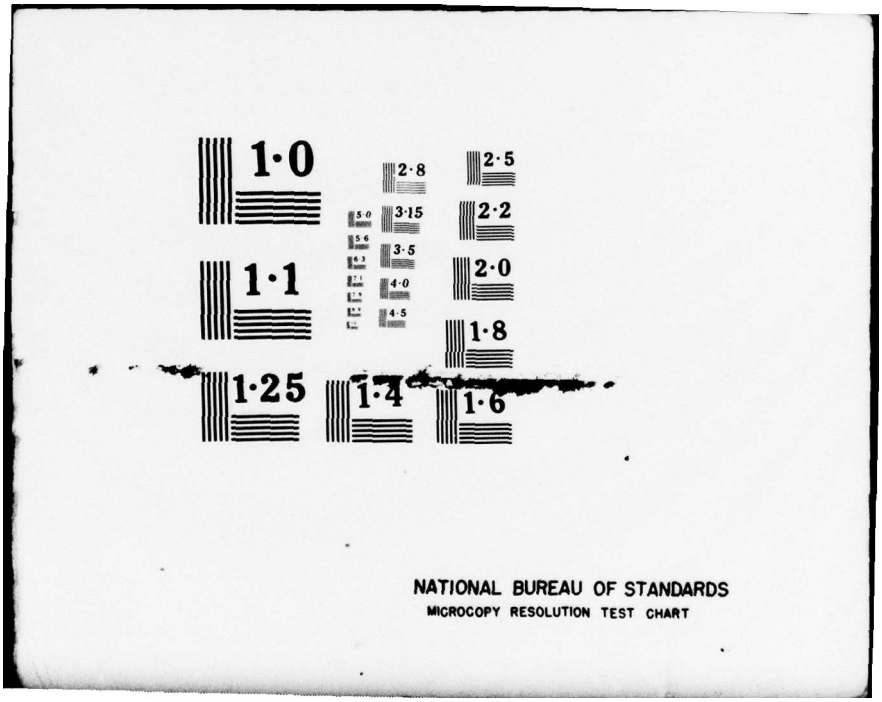
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Small-particle aerosols of rimantadine administered continuously beginning 72 h postinfection for 4 days (21 mg/kg/day) and ribavirin administered beginning 6 h postinfection for 80 min daily for 4 days (26 mg/kg/day) were used to treat experimentally induced influenza A/NJ/8/76 ($H_{sw}1N1$) infection in adult female mice [Dub:(ICR)]. Over a 12-day period following inoculation, mice from each group were studied at random to assess rectal temperature; arterial blood pH, $P_{a_{O_2}}$, $P_{a_{CO_2}}$, and HCO_3^- values; progressive pulmonary pathophysiological (cont'd)

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changes, and concurrent lung lesions and lung virus titers. Results in treated mice were compared with data from control groups of normal and infected untreated mice. The influenza infection with A/NJ virus resulted in hypothermia, bronchial pneumonia, and blood gas alterations. Treatment with ribavirin completely prevented these alterations from occurring. Although rimantadine did not prevent all pathophysiological alterations, it resulted in decreased recovery time.

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Running Head: EFFECT OF RIMANTADINE AND RIBAVIRIN ON SWINE FLU IN MICE

Presented in part at the Federation of the American Society for Experimental Biology, Chicago, Illinois, April 1977.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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ABSTRACT

Small-particle aerosols of rimantadine administered continuously beginning 72 h postinfection for 4 days (21 mg/kg/day) and ribavirin administered beginning 6 h postinfection for 80 min daily for 4 days (26 mg/kg/day) were used to treat experimentally induced influenza A/NJ/8/76 ($H_{SW}1N1$) infection in adult female mice [Dub:(ICR)]. Over a 12-day period following inoculation, mice from each group were studied at random to assess rectal temperature; arterial blood pH, P_{aO_2}, P_{aCO_2} and ~~HCO_3^- values~~; progressive pulmonary pathophysiological changes, and concurrent lung lesions and lung virus titers. Results in treated mice were compared with data from control groups of normal and infected-untreated mice. The influenza infection with A/NJ virus resulted in hypothermia, bronchial pneumonia, and blood gas alterations. Treatment with ribavirin completely prevented these alterations from occurring. Although rimantadine did not prevent all pathophysiological alterations, it resulted in decreased recovery time.

partial pressures of oxygen and carbon dioxide and bicarbonate ion values;

Influenza infections continue to be a major cause of significant respiratory illness, lost time and death in man (3, 4, 8, 13). Much effort towards control of infections has been directed toward the immunization of large segments of the population at risk but with only limited success (2, 10). Hence, there is a demand for effective chemotherapeutic agents and their use may soon be accelerated. Rimantadine hydrochloride (α-methyl-1-adamantane-methylamine hydrochloride) has been proven effective against A strains of influenza virus in animals and man (1, 9, 15). Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) has antiviral activity against both influenza A and B infections in mice (1, 6, 7, 14, 17) and man (5, 12; Magnussen, C. R., R. G. Douglas, Jr., R. F. Betts, F. K. Roth, and M. P. Meagher. Antimicrob. Agents Chemother. in press, 1977). Both compounds have been tested by the aerosol, oral and intraperitoneal routes in rodents and by the oral route in man (1, 16; Magnussen et al., in press, 1977). Effectiveness of the two compounds is apparently related more to time of initiation and duration of treatment rather than to route of administration. The purpose of this study was to examine selected pathophysiologic changes following early initiated ribavirin treatment or late initiated rimantadine treatment on swine influenza adapted to mice.

MATERIALS AND METHODS

Animal. Adult female [Dub:(ICR)] mice were allocated at random 15 per cage and housed in negative pressure hoods after virus exposure. Feed and water were provided ad libitum. Rectal temperatures of mice were recorded before anesthesia and again immediately prior to blood sampling, as previously described (1).

Virus. The mouse-adapted swine influenza virus, A/NJ/8/76 ($H_{sw}1$,N1), was used to infect mice by intranasal (i.n.) inoculation of 10^5 egg median infectious doses (EID_{50}) in 0.05 ml of broth carrier as previously described (Berendt, R. F., and G. H. Scott, J. Infect. Dis., in press, 1977). At this dose less than 5% mortality is expected.

Drug. Rimantadine (E.I. Dupont de Nemours and Co., Inc., Newark, N.J.) and ribavirin (Research Institute, ICN Pharmaceuticals, Inc., Irvine, Cal.) were dissolved in sterile, triple distilled water and given as a small-particle aerosol (SPA) to virus-inoculated mice: (i) rimantadine administered 22 h/day for 4 days beginning 72 h postinfection or (ii) ribavirin given 80 min/day for 4 days beginning 6 h postinfection as previously described (1). Presented doses were calculated to be 21 and 26 mg/kg/day for rimantadine and ribavirin, respectively.

Design. A total of 360 mice were allocated at random into one of four equal groups. Mice in group 1 were uninfected and untreated controls, while those in groups 2, 3 and 4 were infected by i.n. inoculation with swine influenza virus. Mice in group 2 were infected-untreated controls while those in groups 3 and 4 received rimantadine or ribavirin therapy, respectively. Five to eight mice were selected at random from each group to assess the temporal pulmonary pathophysiologic changes on days 0, 3, 6, 7, 8, 10, and 12 postinfection. Variables

measured included rectal temperature, arterial blood pH, arterial oxygen tension ($P_{a_{O_2}}$), arterial carbon dioxide tension ($P_{a_{CO_2}}$), bicarbonate (HCO_3^-), lung lesions, lung/body weight ratio, and lung virus titers. Treatment groups were compared using one- and two-way analyses of variance. Differences were considered significant when $P < 0.05$.

Blood gas tension. Arterial blood samples were obtained from the abdominal aorta of mice under light general anesthesia with 0.5% Halothane as previously described (1). The partial pressures of oxygen ($P_{a_{O_2}}$) and of carbon dioxide ($P_{a_{CO_2}}$) and pH values were measured from heparinized blood samples within 10 min of collection using an automated analyzer (model 165, Corning Instruments, Inc., Medfield, Mass.), calibrated at 37°C. Algorithms of Ruiz et al. (11) were used to correct body temperature and to calculate bicarbonate values.

Lung water contents and lung lesions and lung virus titers. During anesthesia body weight and rectal temperature were recorded. Immediately after blood samples were collected, the thorax was opened and all lung tissue was removed and weighed. The lung-to-body weight ratio was calculated. Lung lesions were scored and lung virus titers assayed in infected controls and in both infected-treated groups using methods previously described (Berendt and Scott, J. Infect. Dis., in press, 1977).

RESULTS

A significant ($P < 0.01$) decrease in rectal temperature occurred on day 7 postinfection in the untreated mice (Fig. 1). Marked hypothermia was not evident in uninfected controls or treated mice (Fig. 1). Lung lesion scores increased significantly ($P < 0.01$) in the infected-untreated

mice beginning on day 6 and peaked on day 7 (Fig. 2). Rimantadine treatment from 3-7 days postinfection did not alter the onset of pulmonary lesions which peaked on day 6; however, lung lesion scores returned toward normal control levels earlier when compared with scores of infected-untreated mice (Fig. 2). Significant lung lesions failed to develop in mice treated early with ribavirin (Fig. 2).

Significant differences in lung virus titers between infected and treated groups were not clearly evident (Fig. 2, inset). However, there was a trend towards decreased lung virus titers when comparing days 3 to 7 in infected-control and rimantadine-treated groups.

Lung-to-body weight ratios were significantly ($P < 0.01$) increased in untreated mice on day 7 postinfection (Fig. 3). Late rimantadine or early ribavirin treatments of infected mice prevented the significant increase in wet lung weights observed in untreated mice (Fig. 3).

Arterial P_{O_2} values of untreated and rimantadine-treated mice decreased to minimum values on days 7 and 6 respectively (Fig. 4a). Subsequently, $P_{a_{O_2}}$ values increased in both groups; however, $P_{a_{O_2}}$ values of rimantadine-treated mice returned toward normal control values earlier than those of untreated mice (Fig. 4a). Infected mice treated earlier with ribavirin failed to show significant alterations in $P_{a_{O_2}}$ values during the course of the study (Fig. 4a).

Significant changes in blood pH were not observed among the four experimental groups (Fig. 4b). Significant increases in both bicarbonate (Fig. 4c) and $P_{a_{C_{O_2}}}$ (Fig. 4d) values occurred in untreated mice on day 7. Early treatment with ribavirin and later treatment with rimantadine appeared to prevent changes in bicarbonate and $P_{a_{C_{O_2}}}$ values when compared to values for uninfected controls (Fig. 4c, d).

DISCUSSION

The pathophysiologic changes associated with experimental, mouse-adapted swine flu virus are characterized by (a) severe hypothermia, (b) increased lung lesion scores, (c) increased lung-to-body weight ratios, (d) ventilation-profusion imbalances as indicated by lowered arterial oxygen tension and elevated arterial carbon dioxide tension, and (e) compensated respiratory acidosis as evidenced by increased bicarbonate and arterial carbon dioxide tension. These findings are strikingly similar to those described for A_2 influenza infections in mice (1). These results also tend to confirm the presence of severe bronchopneumonia with consolidating cellular infiltrates and edema. The morbidity rate of swine flu virus in mice is similar to A_2 influenza, i.e., nearly 100%. However, the high mortality seen with A_2 influenza is not present with A/NJ influenza in mice.

Early therapy with ribavirin (initiated 6 h postinfection) prevented all pathophysiologic changes that occurred in untreated mice. This is consistent with reports that indicate that ribavirin is an effective antiviral chemotherapeutic agent (14). However, a reduction in lung virus titers did not occur during the period of our studies. Late rimantadine therapy (initiated 72 h postinfection) was partially effective in treating A/NJ influenza virus infections. Although rimantadine failed to delay onset of pulmonary lesion, suggesting that it did not alter the ability of the virus to produce some pathologic changes; lung lesion scores in rimantadine-treated mice returned toward normal control values earlier than those in mice from the infected group. The decrease in $P_{a_{O_2}}$ observed in the untreated group was not prevented by rimantadine therapy. However, the $P_{a_{O_2}}$ returned to control values earlier than values

measured in the infected group. Late initiated rimantadine treatment may have sufficiently reduced lung lesions so as to improve blood-gas exchange earlier during the course of infection. The exact mechanisms of action of rimantadine are not known. It has been suggested that rimantadine, an analog of amantadine, may work centrally to prevent respiratory imbalances (1). Late treatment with rimantadine does not completely prevent all pathophysiologic changes associated with A/NJ influenza virus infection in mice. However, rimantadine appeared to have decreased lung lesions sufficiently to prevent the severe alterations found in the infected mice. It is conceivable that a 1-log reduction in lung virus titer at a critical time during infection (i.e., day 7) may be very significant in altering the course of disease and promoting earlier recovery.

One should not conclude that ribavirin is more effective than rimantadine, since treatment schedules and dosages were different. Based upon previous experiments with other strains of influenza virus, the therapy schedules were selected to optimize the chances of showing functional improvements in pulmonary gas exchange.

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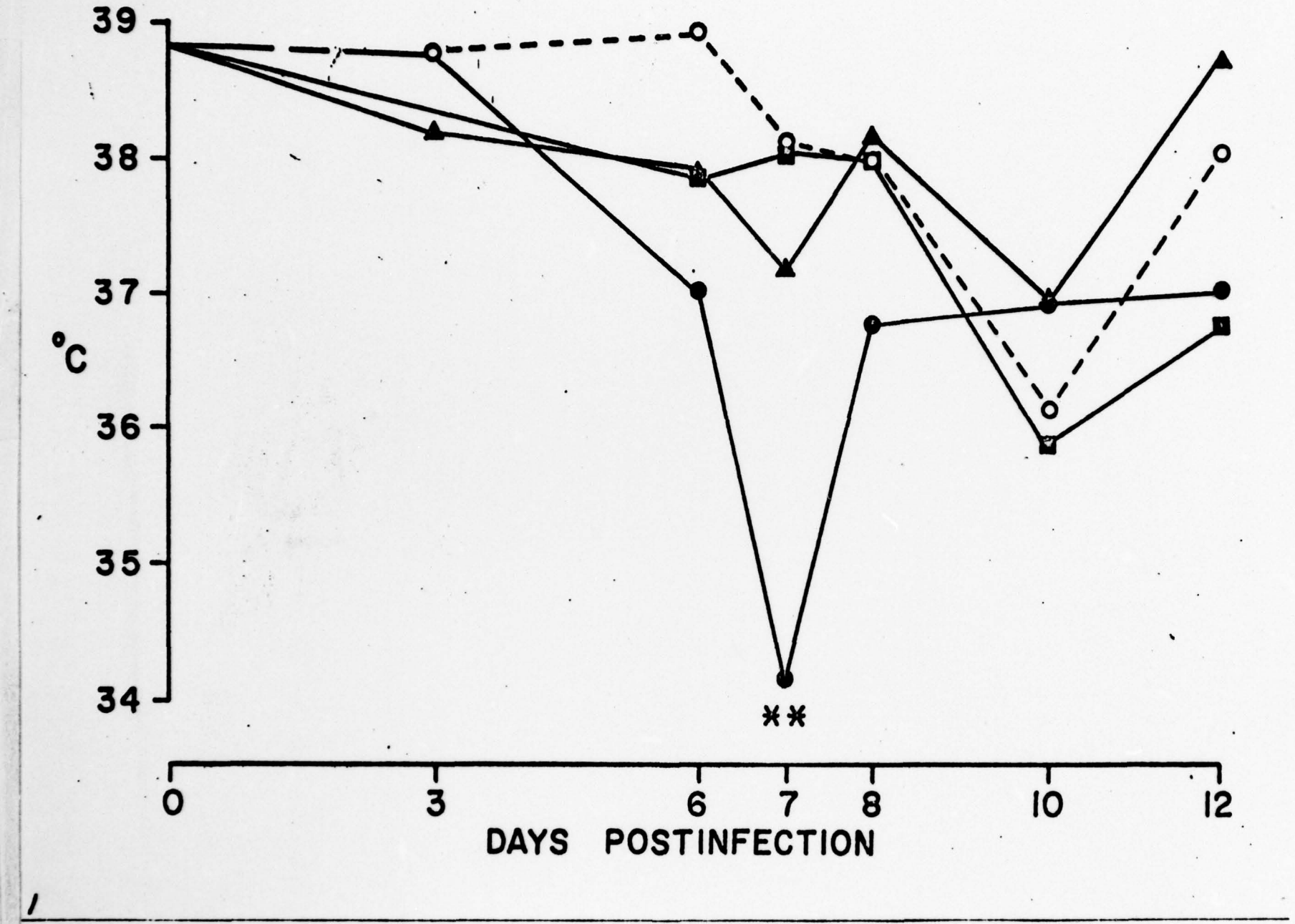
FIGURE LEGENDS

FIG. 1. The effect of rimantadine (▲) and ribavirin (■) on rectal temperature of mice infected with A/NJ influenza virus compared to uninfected (○) and infected controls (●). ** = $P < 0.01$.

FIG. 2. The effect of rimantadine (▲) and ribavirin (■) on lung lesion scores of mice infected with A/NJ influenza virus compared to uninfected (○) and infected controls (●). ** = $P < 0.01$.

FIG. 3. The effect of rimantadine (▲) and ribavirin (■) on lung to body weight ratios of mice infected with A/NJ influenza virus compared to uninfected (○) and infected controls (●). ** = $P < 0.01$.

FIG. 4. The effect of rimantadine (▲) and ribavirin (■) on P_{aO_2} (A), pH (B), HCO_3^- (C) and P_{aCO_2} (D) of mice infected with A/NJ influenza virus compared to uninfected (○) and infected controls (●). * = $P < 0.05$. ** = $P < 0.01$.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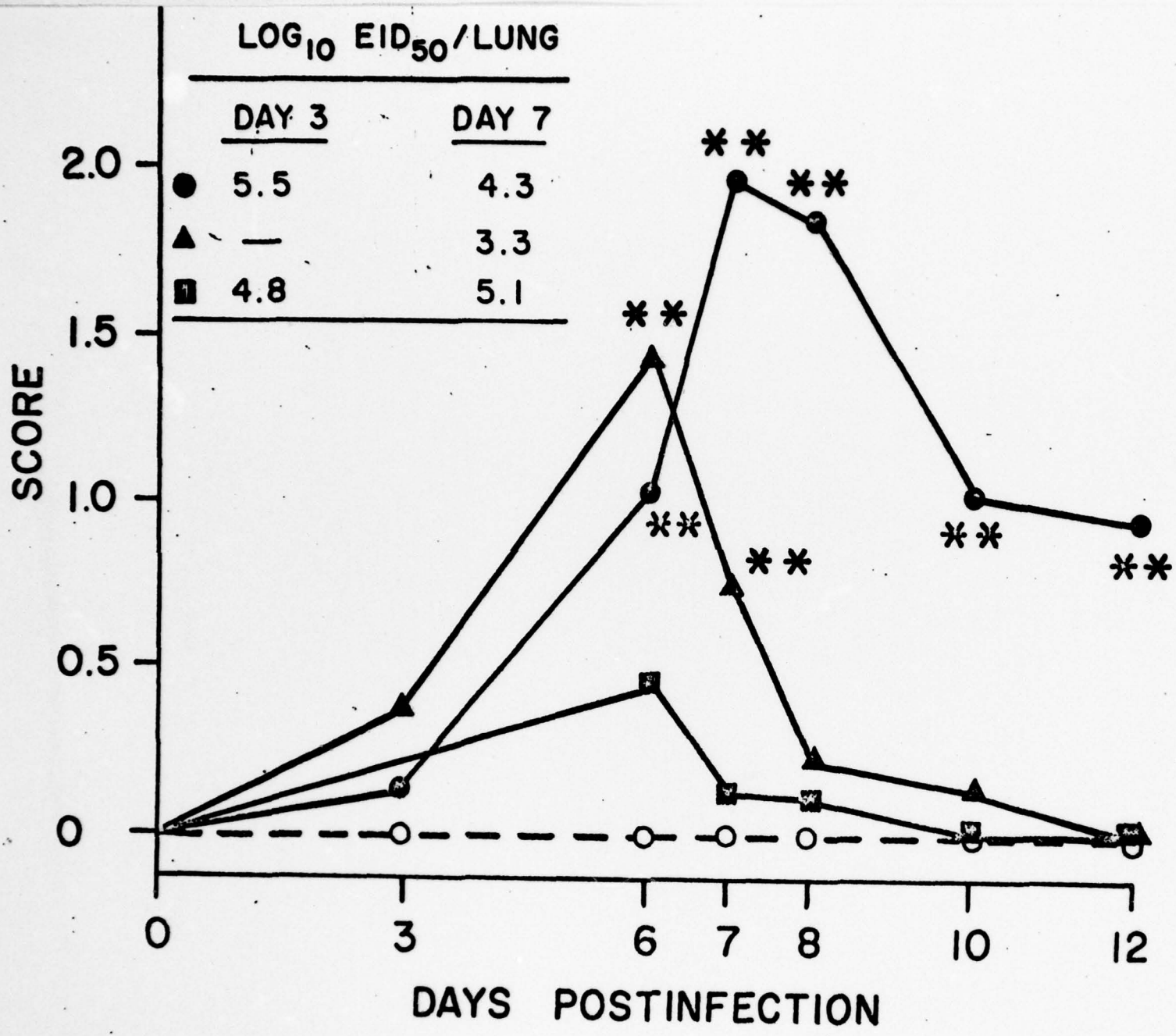
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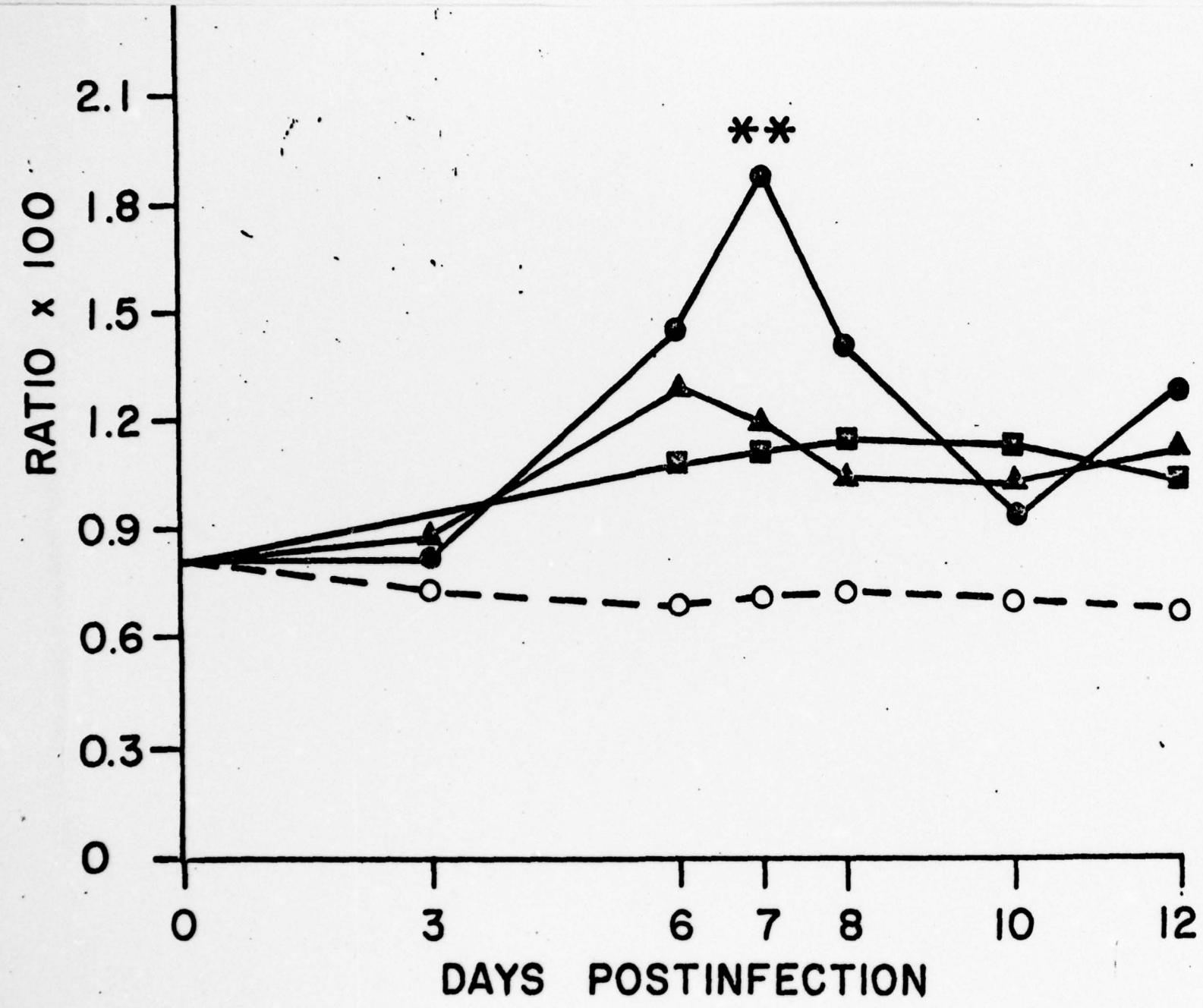
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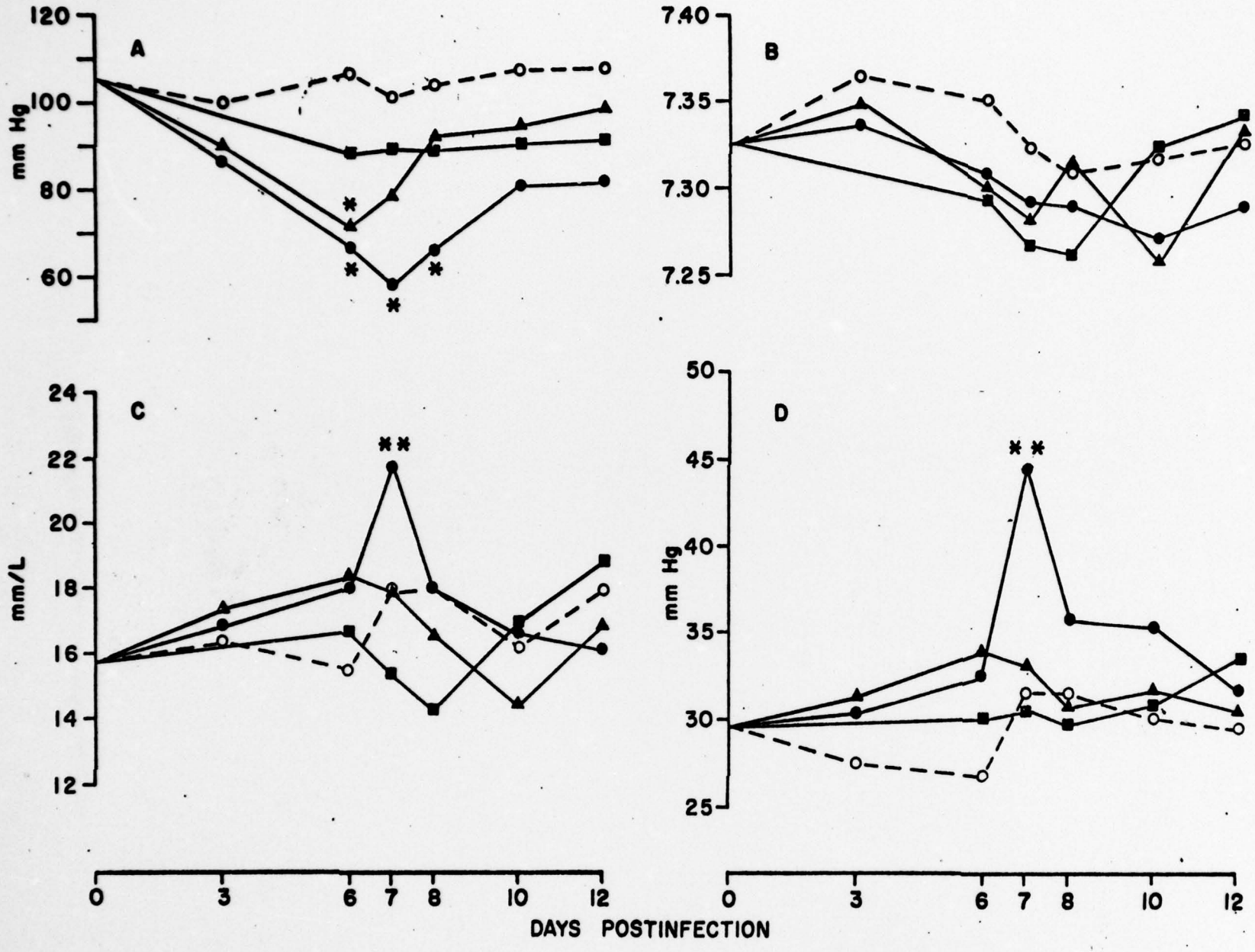
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